Development 126, 2823-2828 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
DEV7703

REVIEW ARTICLE

Patterning mechanisms in the body trunk and the appendages of *Drosophila*

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Accepted 26 April; published on WWW 7 June 1999

SUMMARY

During evolution, many animal groups have developed specialised outgrowths of the body wall, limbs or appendages. The type of appendage depends on the identity of the segment where they appear, indicating that the Hox genes contribute to appendage specification. Moreover, work carried out principally in *Drosophila* has identified the gene products and the mechanisms involved in pattern formation in the appendages. In this essay, we compare the morphogenetic processes in the appendages and the body wall; the function of the Hox genes and the response to the signalling molecules involved in local patterning. We speculate that, although the basic mechanisms are similar, there are significant differences in the manner the body trunk and appendages respond to them.

Key words: Appendage, *Drosophila*, Imaginal disc, Pattern formation

INTRODUCTION

A clear functional and morphological distinction in the body of arthropods is that between the main body wall, which we will refer to as body trunk, and the appendages. The latter are specialised organs sprouting from the trunk, which are generally used for movement. In recent years, we have learnt a great deal about patterning mechanisms in the appendages of *Drosophila* (Basler and Struhl, 1994; Nellen et al., 1996; Lecuit et al., 1996; see Lawrence and Struhl, 1996, for review), but little about the trunk. In this essay, we are going to argue that, although still largely unknown, patterning processes in the trunk differ in several aspects from those in the appendages. Our argument is based on results concerning the adult cuticular patterns of *Drosophila*, but given the conservation of the general design of the body plans in multicellular organisms, we believe it is applicable to other arthropods and may be to vertebrates as well.

The adult cuticle of *Drosophila* is formed by special groups of 'imaginal' cells that do not contribute to the larval patterns. During the larval period, imaginal cells may stay idle, like the abdominal histoblasts (the precursors of the adult abdomen) or may actively proliferate forming sac-like structures called imaginal discs, the precursors of the head, thoracic and genital segments. The imaginal discs can be distinguished by their morphology and the fate of their constituent cells. The mosaic contribution of the imaginal discs and abdominal histoblasts gives rise to the whole of the adult body cuticle (Cohen, 1993; Fristrom and Fristrom, 1993).

One distinctive feature of the thoracic and cephalic discs is that their cells differentiate both body trunk structures and appendages. The second thoracic segment, for example, is made by two pairs of discs, the wing and second leg discs. The wing disc forms mesonotum and pleura, which together form the mesothoracic trunk, and the wing blade (appendage). The leg disc forms the adult leg, which contains trunk as well as appendage components (Fig. 1). Although the trunk and the appendage parts of these segments are easy to discriminate morphologically, the exact boundary between them is not clear. For example, in proximal regions of the wing, there is a smooth continuity with the notum and pleural structures, with no morphological landmark delimiting the two regions. Similarly, there is no clear morphological boundary between the leg appendage and the trunk region (ventral pleura) where it is inserted. This is also reflected in the imaginal discs, which show no morphological distinction between the two regions where fate maps allocate the precursor cells of either component (Bryant, 1978). Moreover, the trunk and appendage primordia may have a common cell lineage throughout most of development. Although a lineage restriction between wing and mesothorax has been described (Garcia-Bellido et al., 1976), no lineage restriction has been found along the proximodistal axis in the leg (Steiner, 1976; Gorfinkiel et al., 1997). As we will argue below, the distinction between trunk and appendage is ultimately based on genetic criteria that are unrelated to morphology or lineage. Trunk and appendages have in common the subdivision into anterior (A) and posterior (P) compartments, a key developmental segregation that generates an interface of cells where critical interactions take place.

MORPHOGENETIC MECHANISMS

Although both the cephalic and thoracic segments contain

appendages, we will center our argument on the thoracic segments since their development is better known. There are two critical components that determine the characteristic morphology of the body segments.

(1) The general patterning mechanism

This involves the function of *engrailed (en)* and the subsequent production of the morphogenetic signals Hedgehog (Hh), Wingless (Wg) and Decapentaplegic (Dpp) (reviewed in Lawrence and Struhl, 1996). The Hh protein behaves as a short-range morphogen that diffuses from the P to the adjacent A cells where it activates genes encoding long-range signals like Dpp (in the wing and haltere discs) or Dpp and Wg (in the leg discs). In addition, Hh has a Dpp-independent patterning role in the proximity of the A/P border (Mullor et al., 1997; Strigini and Cohen, 1997). These signals originate from a narrow region close to the interface between A and P cells and act as organisers to pattern the different structures (Brook et al., 1998). In the wing blade, Dpp acts as a principal morphogen along the anteroposterior axis. Response genes such as *spalt (sal)*, *optomotor-blind (omb)* and *vestigial (vg)* are activated in distinct regions according to the local concentrations of Dpp (Nellen et al., 1996; Lecuit et al., 1996; De Celis et al., 1996; Kim et al., 1997). Similarly, diffusion of Dpp and Wg from the vicinity of the A/P border in the leg disc activates the response genes *Distal-less (Dll)*, *dachshund (dac)* and *omb* in different regions (Díaz-Benjumea et al., 1994; Brook and Cohen, 1996; Lecuit and Cohen, 1997). Thus, the local concentrations of the Dpp and Wg morphogens represent positional readings with respect to their source(s) of origin. These signals establish the set of co-ordinates that define positional information in the disc and are reiterated in the dorsal (wing and haltere) and ventral (the three leg) discs.

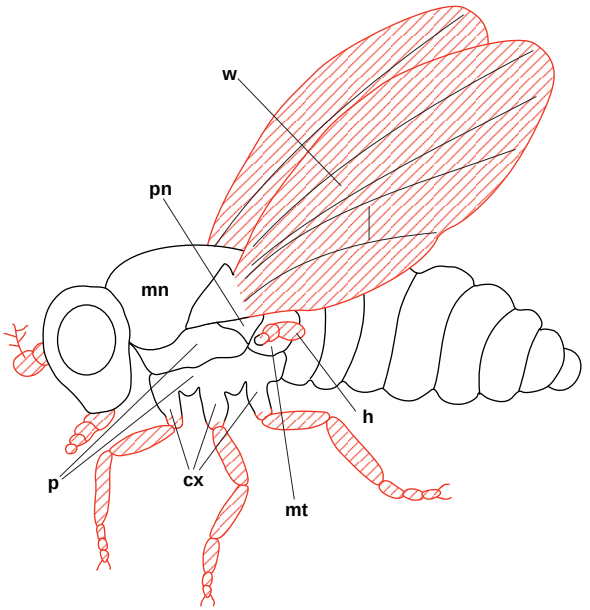

Fig. 1. Scheme of a *Drosophila* adult showing appendages (red hatching) and body trunk (blank). Note that the coxa (cx), usually considered part of the leg appendage, is not considered as such here (see text). w, wing; h, haltere; mn, mesonotum; pn, postnotum; mt, metanotum; p, pleura; cx, coxa.

(2) The function of the homeotic genes

They generate segmental diversity by their specific response to the positional cues established by the morphogens. Two examples may illustrate the point: the disposition of Dpp and Wg is identical in the three leg discs, yet these differentiate distinct patterns as specified by *Sex comb reduced (Scr)*, *Antennapedia (Antp)* or *Ultrabithorax (Ubx)* (Struhl, 1982); in the wing and haltere discs, the presence of the Ubx product in the haltere disc results in a different response to the same Dpp gradient (Weatherbee et al., 1998).

DIFFERENT MORPHOGENETIC PROCESSES IN TRUNK AND APPENDAGES

The basis of our argument is that the trunk and the appendages respond differently to Hh/Dpp/Wg signalling and to *Hox* function.

Patterning mechanisms

A fundamental property of the imaginal discs is that they are subdivided into compartments (Garcia-Bellido et al., 1973). The A/P subdivision is established during embryogenesis (Steiner, 1976; Lawrence and Morata, 1977; Vincent and O´Farrell, 1992), so that when the discs individualise they are already made of A and P compartments. All the cells of the P compartments contain *en* activity (Kornberg et al., 1985). There are two distinct, though related, aspects of the function of *en* that have been studied in detail in the wing disc (Zecca et al., 1995; Tabata et al., 1995; Guillén et al., 1995): one is its role as a selector gene specifying P compartment development and the second is its patterning role inducing *hh* activity that triggers the signalling cascade described above.

Regarding the selector role specifying P compartment development, the overall results suggest that trunk and appendages respond similarly to the loss or gain of *engrailed* function. However, in the wing disc, there are some observations that may suggest a distinction between trunk (mesothorax) and appendage (wing blade). Clones of cells lacking entirely *en* and *invected (inv)* functions in the P wing show a transformation into A wing (Hidalgo, 1994; Tabata et al., 1995), but these clones would be expected to produce a parallel transformation of postnotum into notum that has never been reported – this transformation would have easily been scored. It might be that these clones die or sort out in the trunk (this would already be a difference between trunk and appendage), or else that they do not suffer a comparable transformation. A similar but weaker argument is that viable *en* mutants show a mirror-image transformation of posterior into anterior wing blade (García-Bellido and Santamaria, 1972; Morata and Lawrence, 1975; Lawrence and Morata, 1976), but the same flies fail to show a comparable transformation in the mesothorax. However, in the abdominal segments, a trunk region without appendages, en^{-} inv^{-} clones in the P compartment show a transformation into the corresponding A compartment, whereas *en*-expressing clones in the A compartment show the opposite transformation (Lawrence et al., 1999).

Regarding its role activating *hh*, experiments inducing ectopic expression of *en* suggest a differential response in the anterior compartments of wing and mesonotum. In the A wing compartment, *en*-expressing cells induce local duplications

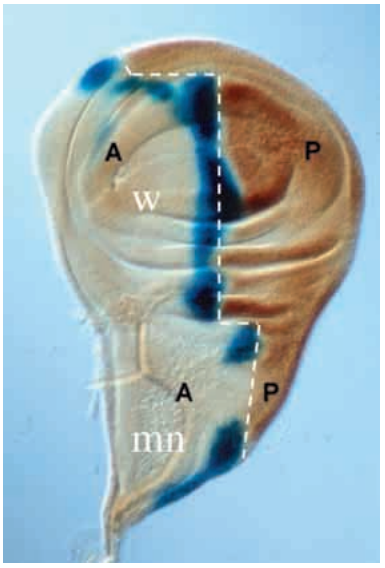

Fig. 2. Wing disc of a *dpp-lacZ Drosophila* larva stained with anti-engrailed antibody (brown), which marks the posterior compartment, and X-Gal, which shows the expression of *dpp*, close to the anteroposterior compartment boundary (marked by a dashed white line). A, anterior compartment; P, posterior compartment. Note the differences in size between A and P compartments in the wing blade (w) and mesonotum (mn).

because they activate *hh* which triggers the organiser function of Dpp (Capdevila and Guerrero, 1994; Ingham and Fietz, 1995; Zecca et al., 1995) but, in the mesonotum, *en*-expressing cells have not been reported to induce such local duplications. This observation suggests that Hh/Dpp signalling is used differently in these two regions (see below).

This idea that the response to Hh signalling is different in trunk and appendages is supported by experiments in which the activity of Hh, Dpp or Wg is changed during imaginal disc development. For example, in the leg disc, the elimination or reduction of either *hh* or *dpp* or *wg* (Díaz-Benjumea et al., 1994; Gonzalez-Crespo and Morata, 1996) results in the loss of the appendage, but no effect is seen in the proximal, thoracic, region. Similarly, in the wing disc, the elimination of *hh* removes the wing but at least some mesonotal regions remain (F. Diaz-Benjumea, personal communication). Moreover, ectopic activity of *dpp* also affects differently the wing blade and the mesothorax; while it produces pattern duplications in the wing, in which large regions are affected, its ectopic activity in the mesonotum only induces supernumerary bristles (Mullor et al., 1997; Tomayasu et al., 1998).

In the idea that Dpp and Wg are major effectors establishing positional cues in the imaginal discs, the conclusion is that the positional co-ordinates that they establish are different in the trunk and in the appendages. In fact, there is a marked difference in the manner that the Dpp and Wg proteins are arranged in the mesothorax and wing blade components of the wing disc. The Dpp signal originates in both regions at the A/P border, from where it diffuses and patterns the A and P compartments (Fig. 2). In the wing blade, the A/P border is located right in the center, subdividing it into the A and P compartments which are approximately of the same size. This results in similar values of morphogen in symmetric locations at each side of the A/P border. Not surprisingly, the elimination of *en* function, which is responsible of the difference between A and P wing compartments (Morata and Lawrence, 1975; Lawrence and Morata, 1976), produces two similar A-like patterns in mirror-image fashion. For the same reason, *en* mutant clones differentiate in the P compartment as in the corresponding, and approximately symmetric, position in the A compartment. In contrast, in the thoracic region, the P compartment (postnotum) is much smaller than the A compartment (notum). This is already noticeable in discs showing *en* or *dpp* expressions that mark the position of the A/P border (Fig. 2). Assuming that Dpp diffuses equally, positional cues must be very different in the two trunk compartments. This may be the reason of the inability to detect postnotum-to-notum transformation in *en* mutant clones.

Moreover, Dpp-response genes, like *omb* or *vestigial (vg)*, that are induced in wing pouch, are not induced in the mesonotum, even though *dpp* is also expressed close to the A/P border. In the leg disc, Dpp- and Wg-response genes, like *Dll*, *dac* and *omb*, are only expressed in the distal region, although there is evidence that the two morphogens are present in the entire disc (Basler and Struhl, 1994; González-Crespo et al., 1998).

The expression and function of *wg* is also very different in the wing blade and the mesonotum. During early disc development, *wg* function is required precisely to allow appendage development and has no role in the mesonotum (Ng et al., 1996); the loss of this function affects only the wing blade, which is transformed into mesonotum. Later, *wg* is expressed and required in the D/V compartment border (Phillips and Whittle, 1993; Couso et al., 1994), but there is no comparable expression or requirement in the homologous compartment border in the trunk (mesonotum/mesopleura). Conversely, there is a late expression of *wg* in the mesonotum, shaped as a longitudinal thin stripe (Phillips and Whittle, 1993). This expression, however, is exclusive to the A compartment, in contrast to that in the wing blade where it is symmetric to the A and P compartments. This trunk domain of *wg* reflects a function with no parallel in the wing blade.

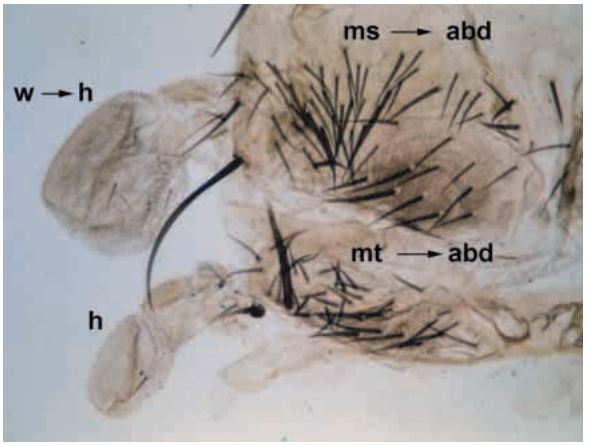

Fig. 3. Left half of a *Drosophila* thorax of genotype C-765/UAS-abdA. In these flies, the Gal4 driver C-765 directs an uniform expression of the UAS-abd-A construct; they show different segmental transformations in body trunk and appendages. The trunk regions, mesonotum (mn) and metanotum (mt), are transformed into abdomen (abd), whereas the wing appendage (w) is transformed into haltere. The haltere (h) remains normal.

Differential response to Hox gene activity

The activity of the *Hox* genes is responsible for segment diversity along the anteroposterior body axis in *Drosophila*. As proposed by Stern (1968) many years ago, the *Hox* (then homeotic) genes modify the cellullar response to the same positional values, thus generating morphological diversity in similar positional fields. Notice how this old proposition is supported by modern findings in that the disposition of the Hh, Dpp and Wg morphogens, which constitute the molecular basis of positional information, is reiterated in the body segments. All thoracic segments exhibit the same distribution of these products (except that Wg is not present in the posterior haltere, Weatherbee et al., 1998). In turn, the abdominal segments also exhibit a similar distribution of Wg and Dpp, although different from that of the thoracic segments (Shirras and Couso, 1996; Struhl et al., 1997). *Hox* activity is required for segment identity of both larval and adult patterns and the effects of *Hox* mutations in larvae and adults correlate well (Duncan, 1987; Kaufman et al., 1990), indicating a similar way of function.

However, in the specification of adult patterns, there is an important difference between trunk and appendage. Consider the *Ubx* gene for example; the conventional view is that *Ubx* specifies the characteristic development of the metathoracic segment, which includes the haltere appendage and the metanotum, a piece of featureless trunk just behind the postnotum (see Fig. 1). This view is based on two arguments. (1) In *Ubx* mutations, the metanotum and the haltere become transformed into mesothorax and wing respectively, producing spectacular four-winged flies (Lewis, 1963). (2) Mutations that induce inappropriate activity of *Ubx* in the wing segment transforms it into a haltere (White and Akam, 1985; Cabrera et al., 1985), producing a fly with four halteres and no wings.

Some recent results, however, call for a qualification of this view. These come from experiments in which *Ubx* and other homeotic genes like *abdominal-A (abd-A)* and *Abdominal-B (Abd-B)* are expressed ectopically in the mesothorax and wing. The experiments made use of the GAL4/UAS method (Brand and Perrimon, 1993), so that different forms of UAS-*Ubx*, UAS-*abd-A* and UAS-*Abd-B* constructs were under the control of various Gal4 drivers specific to different regions of the disc. The expectations were the following. *Ubx* is known to specify both the T3 and A1 segments depending on the amount of product (Smolik-Utlaut, 1990) or the time of expression (Castelli-Gair and Akam, 1995) or both, so ectopic *Ubx* expression in the second thoracic segment (T2, mesothorax plus wing) may produce either third thoracic (T3, metathorax plus haltere) or first abdominal segment, A1; some Gal4 lines might produce T3 and others A1. Using the same rationale, the Abd-A protein should produce a transformation into any of the A2-A4 abdominal segments, whereas the Abd-B product is expected to specify abdominal patterns of A5-A8 types. The key result is that *Ubx*, *abd-A* and, to a lesser extent, *Abd-B*, produce the same transformation in the wing: it is transformed into a haltere-like appendage (Casares et al., 1996). This is in sharp contrast with the effect of the same genes in the mesothorax: *Ubx* induces T3 or A1, and *abd-A* induces a distinct transformation into a more posterior abdominal pattern. The conclusion is that the BX-C genes are highly specific in the mesothorax but not in the wing. In other words, they do not specify the segmental identity of wings and halteres. In wild-type flies, *Ubx* prevents wing formation in the haltere, but only because it is the only Hox gene expressed there. Should *abd-A* be expressed in the haltere, it could do the same job. The lack of specificity of *Hox* function in the wing is emphasised by the observation that not only the Ubx, abd-A or Abd-B products can transform the wing into a haltere-like appendage, but the mouse *Hoxd-11* (an *Abd-B* homologue) can induce the same transformation (N. Azpiazu and G. M., unpublished).

In the ventral appendages, the legs and antennae, the situation may be different, as genes like *Scr* or *Ubx* induce specific appendage identities (Struhl, 1982), although experiments to replace, say, Ubx by abd-A, similar to those performed in the haltere and wing have not been reported. In this context, we wish to emphasize the role of Hh signalling in appendage development and maybe in its identity. It plays a critical role in setting apart the appendage primordia in embryogenesis (Cohen, 1993) and is required for the growth of the appendage (Diaz-Benjumea et al., 1994). Some Hh-/Dpp-/Wg-response genes like *Dll* (in the leg) and *vg* (in the wing) may play an important role in appendage specificity. In the case of *Dll*, it has been shown that it may specify either leg or antennal development depending on the local context (Gorfinkiel et al., 1997), and it seems that *vg* may also specify wing or haltere depending on the presence or absence of the Ubx product (Maves and Schubiger, 1998). One might speculate that developmental specificity in the appendages depends on a combination of Hh-response and Hox genes; the latter may just be cofactors of the primary function of *Dll* or *vg*. A similar idea has recently been proposed based on the examination of Hox response genes in wings and halteres (Akam, 1998).

THE ROLE OF *extradenticle*

A factor that may be responsible for the difference in *Hox* function in trunk and appendages is *extradenticle (exd)*, itself another homeobox gene, which acts as a cofactor of *Hox* genes. There is ample evidence, molecular and genetic, that *exd* is a critical element contributing to the specificity of *Hox* genes (Peifer and Wieschaus, 1990, see review by Mann, 1995). *exd* has a peculiar mode of regulation: its product is nuclear (as expected for a homeodomain protein) where the gene is functional, and it is cytoplasmic where the gene is not required, so its function is regulated at the post-translational level (Mann and Abu-Shaar, 1996; Aspland and White, 1997). Mosaic analyses inducing marked exd^{-} clones in different body regions (González-Crespo and Morata, 1995; Rauskolb et al., 1995) have shown that *exd* function is not necessary in the distal regions of wings, halteres and legs. The only appendage where it appears to be needed is the antenna, where *exd* has a specific developmental role of its own (Casares and Mann, 1998).

Expression studies fit with the map of functional requirements; the Exd product is nuclear in cells of the trunk and in the base of the appendages (except in the antennae), but not in the distal appendages (Mann and Abu-Shaar, 1996; Aspland and White, 1997). The implication of this is that the homeotic proteins act in the appendages without the concourse of Exd (although one cannot exclude the possible existence of other cofactors), and their function may not be specific in some cases. At this point, it is worth mentioning that in vitro studies have shown that by themselves different homeotic products bind to a number of DNA sites with similar specificity and affinity (reviewed in Hayashi and Scott, 1990). In contrast, the

protein complex Hox/Exd exhibits much higher DNA-binding specificity (reviewed in Mann, 1995). Thus the lack of specificity of Hox proteins in the wing may simply reflect the lack of nuclear Exd protein. It has been suggested that, in isolation, the Hox products act as transcriptional repressors, but in association with Exd form complexes that function as activators (Biggin and McGinnis, 1997). This general, and possibly non-specific, repressor role could account, at least in part, for the lack of Hox specificity in the wing.

Why is *exd* function lacking in the appendages? The explanation may reside in recent findings in the leg disc. This disc is subdivided into two distinct regions according to the requirements and function of the Hh signalling cascade. In the distal region, there is full expression of the Hh cascade, and all response genes such as *Dll*, *dac* and *omb* are activated. Some of these genes like *Dll* are critical for the normal growth and patterning of the leg and may also serve a similar function in all arthropods (Cohen and Jurgens, 1989; Gorfinkiel et al., 1997; Panganiban et al., 1997). The proximal region is defined by the activity of *exd*, whose protein here is located in the nuclei of cells. The function of *exd* prevents the activation of some Hh-response genes, like *omb* or *dac*, so in fact *exd* prevents the full expression of the Hh cascade (although not the diffusion of the Dpp and Wg signals). Thus the functioning of the Hh cascade requires the elimination of *exd* activity. This is achieved by *Dll*, and maybe other response genes, which prevents Exd nuclear translocation, very likely through suppression of *hth* activity. *Dll* performs this role already during the embryonic period (González-Crespo et al., 1998): at embryonic stage 14, *Dll*-expressing cells are the only ones in the thoracic segments in which Exd is cytoplasmic. The activation of *Dll* during the embryonic period also responds to Hh (through Wg, Cohen, 1993), so it seems that one of the first steps to generate an appendage is the elimination of *exd*, in order to allow complete response to Hh signalling. In the wing disc, there is a similar situation: the Exd protein is cytoplasmic in most of the wing blade, while mainly nuclear in the mesonotum. The factors regulating Exd subcellular distribution in the wing disc have not been identified, but we would expect a Hh-/Dpp-response gene to be involved.

The result of the mutual antagonism between *exd* function and Hh signalling is that legs, wings and halteres can only develop in the absence of *exd*, which is a critical factor to confer specificity to *Hox* function. Consequently, different Hox proteins may exhibit similar effects on appendages. Thus, the lack of *exd* activity, and hence of Hox specificity, may be a consequence of the functioning of the Hh cascade.

Given the correspondence between *exd* function and subcellular distribution, Hh signalling and Hox specificity, the subcellular localisation of Exd may allow a distinction to be made between the true appendage and the trunk. The region containing inactive (cytoplasmic) Exd would represent the true appendage, whose growth and pattern is established by the Hh cascade. The trunk would contain active (nuclear) Exd, and Hh signalling would not operate in the manner that it does in the appendage. This distinction does not correspond with morphological landmarks: in the leg, for example, elements like the coxa and the trochanter look like appendage components, whereas by the definition above are part of the trunk. Using a similar argument, some proximal parts of the wing blade would be part of the trunk. It is worth pointing out that Snodgrass (1935), based on comparative studies in different arthropods legs, proposed a subdivision of the appendage into coxopodite and telopodite, which appears to coincide very well with the two regions defined by *exd* functions and the functioning of Hh signalling.

EVOLUTIONARY CONSIDERATIONS

The Hox complex was already operating in the Cambrian, about 540 million years ago and is responsible for the morphological diversity along the anteroposterior body axis in all animals (Slack et al., 1993). The activity of the Hox genes together with the Exd cofactor ensured the high specificity necessary for the different segment identities and is likely a key factor responsible for the 'Cambrian explosion' that gave rise to all extant types of body plans. Arthropods, and later chordates, developed many different forms of appendages. It is likely that the last common ancestor of these groups bore some primitive body wall outgrowths (Panganiban et al., 1997), similar to the lobopodia found in the sister group of arthropods, the Onychophora. It also seems reasonable that appendages appeared after the diversification of the body wall as specified by the Hox genes. The formation of appendages was an evolutionary innovation that implicated the identification and developmental isolation of the groups of trunk cells that were destined to develop into an appendage. The process also required additional growth. This is the task carried out by the particular form of the Hh cascade described for the appendages. Patterning processes in the trunk appear to be quite different, although they also make use of the same signalling molecules.

We thank Peter Lawrence, Gary Struhl and Fernando Díaz-Benjumea for comunicating results before publication, Ana Macías for providing Fig. 2 and Peter Lawrence, Isabel Guerrero, Jordi Casanova, Fernando Díaz-Benjumea and Walter Gehring for comments on the manuscript. The work has been supported by the Dirección General de Investigación Científica y Técnica, a Human Science Frontiers grant (RG 0374/1997M) Comunidad de Madrid (08.9/0003) and the Fundación Ramón Areces.

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